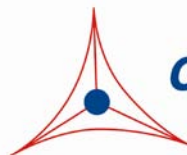

Product Manual

Human Carcinoembryonic Antigen (CEA) ELISA Kit

Catalog Numbers

PRB- 5059	96 assays
PRB- 5059- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Carcinoembryonic antigen (CEA) is a glycoprotein produced in gastrointestinal tissue during fetal development. In healthy adults, serum CEA concentrations are typically at very low levels (< 5 µg/mL); however, many benign conditions (e.g. smoking, IBS, cirrhosis) and intestinal cancers can increase these levels. Because of this, elevated CEA levels are usually only suggestive, and not diagnostic, of colorectal cancers. Nevertheless, CEA remains a useful biomarker/monitoring tool for these tumors and aids in determining their progression, therapy effectiveness, and recurrence.

Cell Biolabs' CEA ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the human CEA protein. The kit has detection sensitivity limit of 150 pg/mL CEA. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and CEA samples.

Assay Principle

An anti-CEA coating antibody is adsorbed onto a microtiter plate. CEA protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-CEA antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-CEA antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of CEA present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified CEA and sample concentration is then determined.

Related Products

1. PRB-5049-C: Human PSA ELISA Combo Kit (Free + Total)
2. PRB-5049-FREE: Human Free PSA ELISA Kit
3. PRB-5049-TOTAL: Human Total PSA ELISA Kit
4. PRB-5058: Human AFP ELISA Kit
5. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
6. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
7. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)
8. CBA-125: Radius™ 24-Well Cell Migration Assay (Microscopy)
9. CBA-126: Radius™ 96-Well Cell Migration Assay (Microscopy)
10. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-CEA Antibody Coated Plate (Part No. 50591B): One strip well 96-well plate.
2. Biotinylated Anti-CEA Antibody (1000X) (Part No. 50592D): One 20 μ L vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 μ L vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human CEA Standard (Part No. 50593D): One 100 μ L vial of 5 μ g/mL human CEA.

Materials Not Supplied

1. CEA Sample: serum, plasma, lysate
2. 10 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
3. 50 μ L to 300 μ L adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store CEA Standard at -20°C and avoid freeze/thaw. Store all other components at 4°C .

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-CEA Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-CEA Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of CEA Standard in the concentration range of 5 ng/mL – 0.078 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	5 µg/mL Human CEA Standard (µL)	Assay Diluent (µL)	CEA (ng/mL)
1	4	3996	5
2	500 of Tube #1	500	2.5
3	500 of Tube #2	500	1.25
4	500 of Tube #3	500	0.625
5	500 of Tube #4	500	0.313
6	500 of Tube #5	500	0.156
7	500 of Tube #6	500	0.078
8	0	500	0

Table 1. Preparation of CEA Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of CEA sample or standard to the Anti-CEA Antibody Coated Plate. Each CEA sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
4. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-CEA Antibody to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.

11. Warm Substrate Solution to room temperature. Add 100 μ L of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

12. Stop the enzyme reaction by adding 100 μ L of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical CEA ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

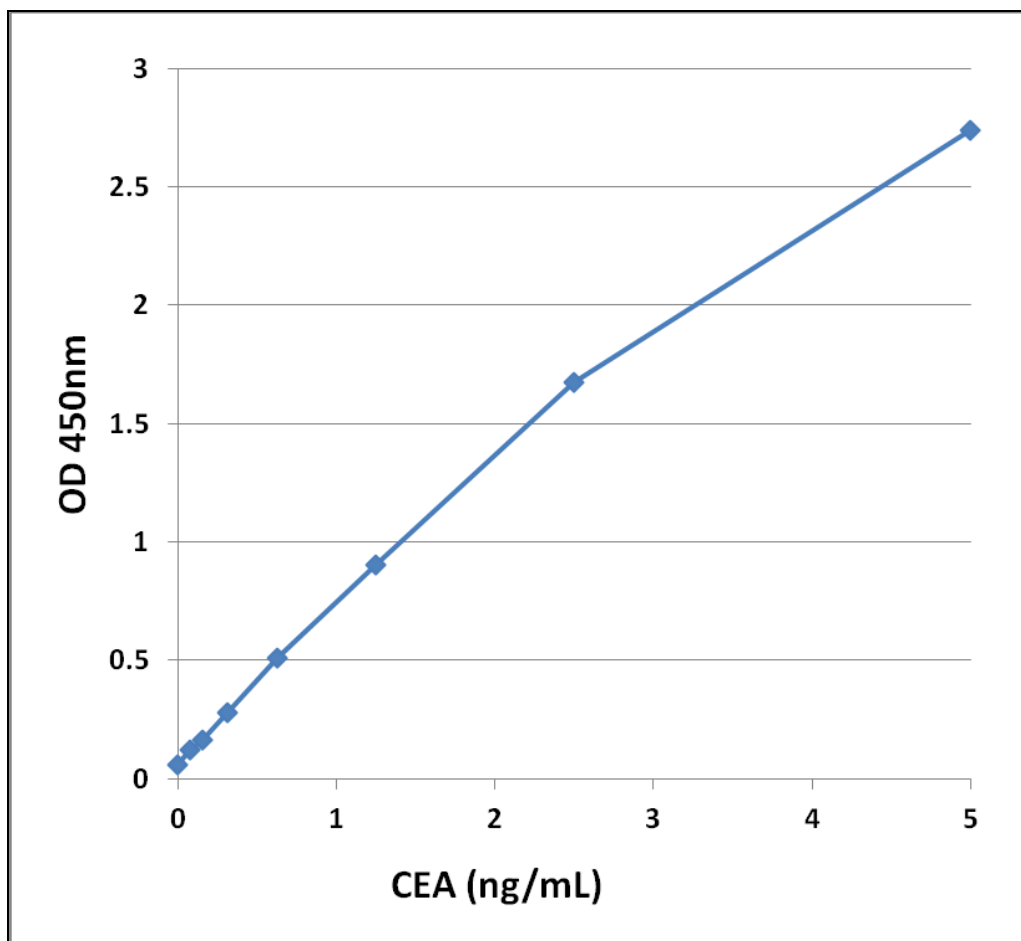


Figure 1: CEA ELISA Standard Curve

References

1. Camunas, J. et al. (1991) *Eur. J. Surg. Oncol.* **17**:530-535.
2. Kuusela, P., C. Haglund, P. Roberts (1991) *Br. J. Cancer* **63**:636-640.
3. Nilsson, O. et al. (1992) *Br. J. Cancer* **65**:215-221.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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